In this Issue

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Neighbourhood Watch in Early Skin Cancer

An emerging view in cancer biology is that the tumor cell microenvironment plays a role in the modulation of neoplastic progression and phenotype. In stratified squamous epithelium, normal cells have the capacity to suppress the early stages of neoplasia in neighbouring cells with malignant potential. Modification of these normal cells by ultraviolet radiation or 12-O-tetradecanoylphorbol-13-acetate (TPA) can abrogate this growth-suppression. Many tumours (including skin tumours) are thought to arise as a result of multiple somatic genetic events that generate a premalignant lesion consisting of cells at varying stages of cancer progression. On page 384, Vaccariello *et al* address whether neighboring cells at an early stage of the transformation process are potentially important in skin tumorigenesis by permitting clonal expansion of cells at a more advanced stage of transformation (perhaps simply by acting as a buffering zone between normal keratinocytes and the more malignant cells).

Using an organotypic model of early neoplasia, in which β-galactosidase-transduced malignant keratinocytes were mixed in three dimensional cultures with normal human keratinocytes or with immortalized HaCaT cells, the authors show that HaCaT cells allowed clonal expansion of malignant keratinocytes, whereas normal keratinocytes were not permissive for expansion. Furthermore, in contrast to normal keratinocytes, which induced filaggrin expression in the malignant keratinocytes, surrounding HaCaT cells were unable to induce differentiation in the malignant keratinocytes. The mechanism for this growth suppression seems to arise from direct cell contact rather than soluble factors secreted by the normal keratinocytes, because malignant suprabasal cells that were growth-suppressed by normal keratinoctyes in normal tissue context were again able to clonally expand in submerged cultures when trypsinised from organotypic cultures and grown in the presence of normal keratinocytes that were not in direct contact with the malignant cells. The use of HaCaT cells (which contain p53 mutations) in the study would seem to be all the more relevant because other workers have shown that human skin chronically exposed to ultraviolet radiation contains multiple clones of p53 mutated cells, in which it is hypothesised that skin cancers later arise.

Disruption of normal tissue architecture by the immortalized HaCaT cells can therefore abrogate the "neighbourhood watch system" employed by normal keratinocytes to control clonal expansion of potentially malignant cells in skin and may perhaps permit the progression of clones of dysplastic, premalignant cells to full blown cancer.

Limitations in Predicting Phenotype in Hereditary Blistering Disorders from Mutation Analysis of Genomic DNA

Molecular analysis of various types of epidermolysis bullosa (EB) has allowed some genotype-phenotype correlations to be made, but as more evidence accumulates it is becoming clear that the molecular pathogenesis underlying these disorders is more complex. For example, mutations in different regions of the keratin 5 or 14 genes were initially thought to account for different clinical subtypes of EB simplex (EBS), and mutations in those areas encoding the ends of the central rod domain, and the helix initiation or termination sites were associated with more severe disease. However, it is now known that each of the three major types of EBS can result from missense mutations at a single identical site.

Similarly, in the more severe forms of recessive dystrophic EB (RDEB) and junctional EB (JEB), both copies of the type VII collagen (COL7 A1) gene or laminin 5 genes (LAMA3, LAMB3, and LAMC2), respectively, have usually contained nonsense or frameshift mutations. Now, McGrath *et al* (p. 314) report on two unrelated families, one with RDEB and one with JEB in which affected individuals exhibit a milder phenotype but contain frameshift and/or nonsense mutations in both alleles of the respective genes. Surprisingly in the RDEB family, although the genomic DNA sequences would have predicted severely truncated type VII collagen, cDNA analysis revealed in-frame skipping of the exon containing the frameshift mutation, resulting in a mildly truncated protein and anchoring fibrils whose function is sufficient to limit the severity of the blistering. In the JEB family, the combination of frameshift/nonsense mutations in LAMB3 resulted in an in-frame deletion of exon 17 in the cDNA from the nonsense allele, and although immunostaining with a β3 chain antibody was completely negative, weak immunopositivity was seen for both the γ2 and α3 chains, consistent with the milder than expected phenotype.

The message emerging from studies such as this is that genotype-phenotype correlations in EB cannot be reliably made on simple sequencing of genomic DNA, but that a combination of techniques is required.

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PUVA: The Long Burn?

Ever since Barry Marshall's discovery of Helicobacter as the cause of peptic ulceration, there seems ever less reason to doubt the maxim that potential major clinical discoveries lie all around us; so often it seems what is required is not only an ability to challenge (or ignore) conventional wisdom but an ability to model the right clinical question experimentally. Peter Farr and Sally Ibbotson on p. 346 offer us one such modest, but important, example.

First a little background. The clinical use of PUVA has been largely empiric with major differences in dosage regimes between different centres. One issue has related to how often the therapy is administered, twice, three or even four times a week. How should we chose? Firstly, and obviously, on the basis of efficacy. But one key trade off that we have to factor in early, relates to side-effects, most notably and obviously burning. How do we take this on board in designing therapeutic regimens? Farr and Ibbotson point out that the kinetics of erythema are critical. If PUVA erythema peaks after say 3 d, dosage regimens more frequent than this may run the risk of cumulative erythema, which might translate as more burning, which in turn will influence compliance. In a straightforward piece of clinical science they show that contrary to previous dogma or ex cathredra statements PUVA erythema peaks at 96 h. A simple conclusion is that phototesting to determine dosage regimens should take this into account. What however, about the frequency of PUVA therapy: should this be reduced to every 96 h? Farr and Ibbotson are rightly more cautious here. The answer is as yet not clear-cut and requires further experimentation. The pattern of accumulation of erythema will be heavily dependent on the order of kinetics of removal of erythema following *multiple* exposures, in particular, any interaction between degree of erythema and speed of removal. Single dose kinetics may provide a model for this or they may not. Second, what is limiting in clinical terms is pain or discomfort rather than erythema. Whether the relation between pain and erythema is constant or context dependent also will require further investigation. There is one other thought for the future: dosage regimens may not only influence immediate toxicity, in terms of burning, but it also becomes ever less fanciful to imagine that these acute inflammatory profiles may influence carcinogenicity. For the present we should note – yet again – that the textbooks are wrong.